

Determining the Hydroxyl Content of Certain Organic Compounds

Macro- and Semimicromethods

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A modification of macroprocedures for determining the hydroxyl content of hydroxylated fatty acids and alcohols is described, in which an internal indicator is used. For colored solutions a potentiometric method has been developed. A semimicroprocedure using an internal indicator is also presented. An acetylating solution of 1 volume of acetic anhydride in 3 volumes of pyridine and a hot-water hydrolysis are used.

THE West, Hoagland, and Curtis (14) modification of the Verley and Bölsing (13) procedure for determining hydroxyl specifies the acetylation of fats and fatlike material with a solution of 1 volume of acetic anhydride in 7 volumes of pyridine, followed by hydrolysis with hot water and titration of the acid formed with alcoholic alkali solution. Peterson and West (10) recommended an acetylating solution of 1 volume of acetic anhydride in 2 volumes of pyridine, and Marks and Morrell (6) adopted the use of a solution of 1 volume of acetic anhydride in 3 volumes of pyridine. In the last two procedures, the excess acetic anhydride is hydrolyzed by adding ice water to the reaction mixture. Malm, Genung, and Williams (5) studied the effects of time, temperature, and concentration of the acetic anhydride in the anhydride-pyridine solution on the acetylation of cellulose derivatives. They found that an acetylation period of 24 hours was required to obtain calculated free hydroxyl values when they used a 0.5 molar acetic anhydride reagent (1 part of acetic anhydride to 19 parts of pyridine). Titrations were conducted electrometrically in an open beaker.

The methods of West, Hoagland, and Curtis and of Marks and Morrell gave low results with hydroxylated higher fatty acids, certain oxidation products of higher fatty acids, and long-chain alcohols analyzed in this laboratory. However, by combining the acetic anhydride acetylating solution of Marks and Morrell with the hot-water hydrolysis and the homogenization with *n*-butanol of West, Hoagland, and Curtis, good results were obtained on the types of material mentioned. Since a concentration of 1 part of acetic anhydride in 7 parts of pyridine gave incomplete acetylation, the more dilute solution of Malm, Genung, and Williams was not investigated. Other work (1, 4, 8, 9, 11, 12) has been described in which both acetic anhydride and acetyl chloride were used for determining the hydroxyl content of organic compounds.

In each of the methods, except that of Malm *et al.*, an internal indicator is employed, which limits the accuracy of analysis of samples producing dark solutions. For these materials a procedure using potentiometric titrations was developed. When the reaction was carried out in the usual manner in an iodine flask, it was necessary to transfer the reaction mixture to a beaker to make the potentiometric titration. During this transfer, small amounts of acetic acid were inevitably lost, introducing relatively large errors. To eliminate these errors, a modified iodine flask was designed that would permit the electrometric titration to be made in the reaction vessel when a Beckman pH meter equipped with extension electrodes is used.

This flask (Figure 1) was made by sealing side arms containing No. 16 standard taper stoppers on opposite sides of a 250-ml. flask. The side arms allow the tips of the electrodes to be immersed in the solution without touching the bottom of the flask.

A semimicromethod using the same reagents employed for the macrodetermination was developed for analyzing samples too small for the macroprocedure. A glass-stoppered, pear-shaped flask (Figure 2) was designed so that the small volumes of the sample and of the reagent could be held in the conical tip, ensuring complete mixing. A 50-ml. flask provides sufficient volume for the addition of the titrating solution. Four tenths milliliter of the acetic anhydride-pyridine solution (1 to 3) is used, since this quantity is sufficient for complete acetylation and also require less than 25 ml. of 0.1 N alkali for neutralization. This volume of reagent permits the use of a 25-ml. buret (calibrated), which deliver a measured volume with the necessary accuracy.

The acetylating reagent is measured from an S-shaped capillary buret of 1-mm. bore (Figure 2) having a mercury

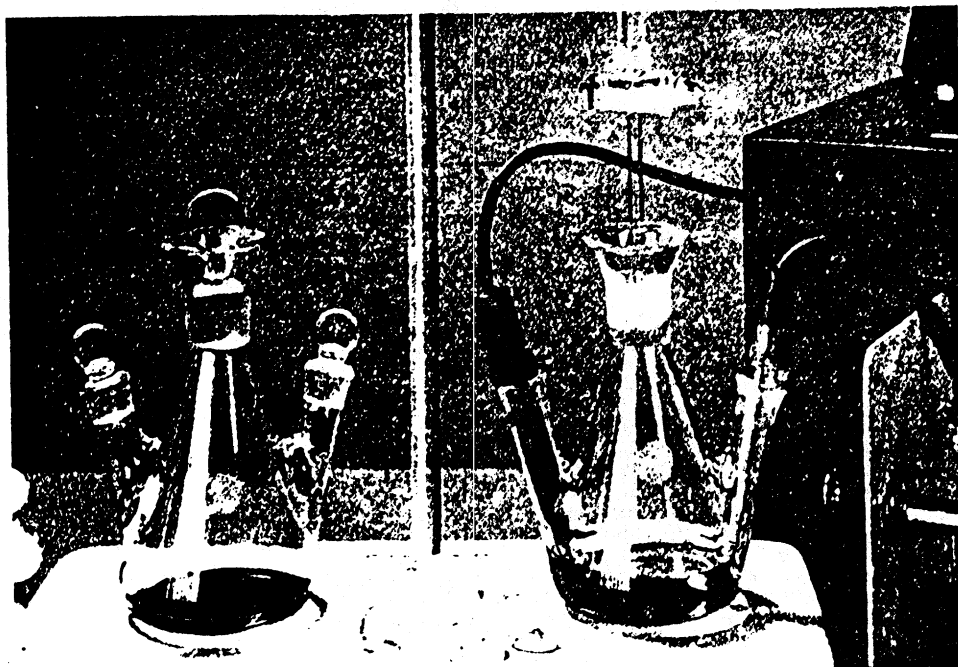


Figure 1 Modified Iodine Flask for Potentiometric Titrations

column in contact with the solution. The buret is filled and the reagent expelled by releasing and applying pressure to the rubber tubing which serves as the mercury well of the buret. This is done by manipulating two screw clamps, *A* and *B*, in contact with the rubber tubing. Since a fixed volume of the acetic anhydride-pyridine reagent is to be delivered, two hair lines are placed on the buret at the points that would allow the delivery of approximately 0.4 ml.

REAGENTS

Acetylating reagent. One volume of A.C.S. grade acetic anhydride mixed with 3 volumes of reagent grade pyridine, *n*-Butanol (Eastman Kodak, practical).

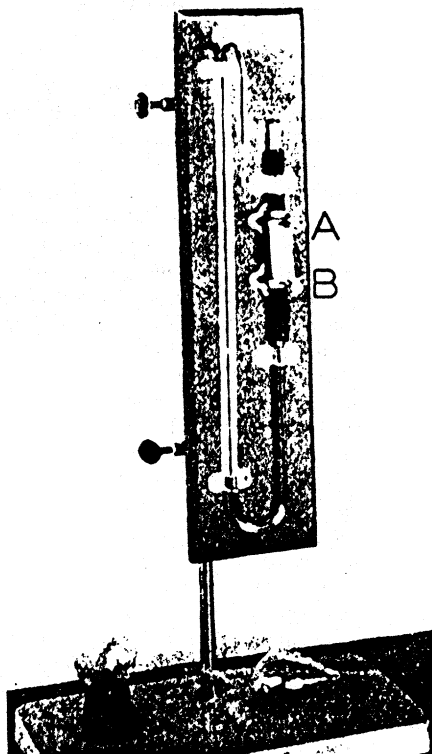


Figure 2. Capillary Buret and Semimicro Reaction Flasks for Semimicro method

Mixed indicator solution (4). One part of 0.1% aqueous solution of cresol red neutralized with sodium hydroxide and 3 parts of 0.1% thymol blue neutralized with sodium hydroxide.

Standard alcoholic sodium hydroxide solutions. Macroprocedure, approximately 0.5 *N*. Semimicroprocedure, approximately 0.1 *N*.

Prepare the solutions from saturated aqueous sodium hydroxide solution (approximately 18 *N*) and aldehyde-free ethanol made by alkaline aluminum reduction. Standardize the alcoholic solutions to ± 2 parts per 1000 against either potassium acid phthalate or a standard acid of approximately the same normality, using the mixed indicator.

MACROPROCEDURE

Place a weighed sample containing from 1 to 2.5 milliequivalents of hydroxyl in the modified iodine flask and add exactly 3.00 ml. of the acetic anhydride-pyridine solution (1 to 3) from a reservoir-type 5-ml. microburet with a Drierite protecting tube in the reservoir. Moisten all stoppers of the flask with pyridine and place the two stoppers firmly in the electrode arms. The center, or main stopper, should be loosely seated. Place the flask on a steam bath. After heating for 45 minutes, add 5 to 6 ml. of water to the cup of the flask and loosen the stopper in such a manner as to rinse the stopper and inside walls of the flask. Continue heating for 2 minutes and then cool under the tap with the main stopper partly removed. With 10-ml. of *n*-butanol rinse the three stoppers and inside walls of both the flask and side arms. Insert the glass and calomel electrodes in the side arms and ti-

trate with 0.5 *N* alcoholic alkali to pH 9.8 (volume *A*). Make a blank determination (volume *B*) on 3.00 ml. of the acetic anhydride-pyridine solution.

Determine the free acid of the sample by repeating the procedure described above, using pyridine instead of acetic anhydride-pyridine solution and adding 5 ml. of neutral ethanol just prior to the titration to make the solution homogeneous. Shake well and titrate with 0.5 *N* alcoholic alkali. Calculate the volume of alkali required to neutralize the acidity of 1 gram of the sample (volume *C*).

CALCULATION.

$$[B - (A - C \times \text{wt. of sample for OH determination})] \times \frac{N \times \text{OH}}{1000} \times 100 = \frac{\text{weight of sample for OH determination}}{\% \text{ OH}}$$

Because of the ease of manipulation, it is recommended that for colorless solutions ordinary iodine flasks and the mixed indicator be used with this procedure instead of the special flasks and the potentiometric titration.

SEMIMICROPROCEDURE

Weigh a sample having a hydroxyl content equivalent to approximately 2 ml. of 0.1 *N* alkali directly into the dry, pear-shaped reaction flask. Draw the acetic anhydride-pyridine reagent into the S-shaped buret to a point below the lower hairline. Wipe the tip of the buret with a towel and then with the fingertips to deposit a slightly oily film, which ensures a more uniform removal of the reagent from the tip. Hold the inside wall of a beaker against the buret tip and bring the mercury meniscus to the lower mark by carefully closing the screw clamp, *A*. Replace the beaker with the reaction flask held so that the buret tip touches the inner wall, and rotate the flask as the reagent is slowly discharged from the buret by tightening *A* and then *B* until the meniscus is even with the upper mark. Immediately connect the flask to a water-cooled condenser and seal the glass joint with a few drops of pyridine. Place the flask and the condenser on a steam bath with the tip of the flask extending approximately 1.25 cm. (0.5 in.) through a tightly fitting ring. Acetylate for 30 minutes, add 3 ml. of distilled water through the condenser, and hydrolyze by heating for 2 minutes longer if no carboxyl groups are present or 30 minutes longer if the sample contains organic acids.

Add 1 ml. of pyridine to the cup and disconnect the flask in such a manner that the pyridine rinses the condenser tip. Loosely insert a stopper and immediately cool the flask to room temperature under running water. Add 3 ml. of *n*-butanol to the cup and loosen the stopper so that the stopper and the walls of the flask are rinsed. Add 3 drops of the mixed indicator and cover the flask with a rubber dam, held in place by a rubber band, to prevent the contents from absorbing carbon dioxide from the air near the end of the titration. Insert the buret tip through a pin-hole in the dam and titrate the excess of acetic acid and any acid in the sample with 0.1 *N* alcoholic sodium hydroxide until the solution changes to gray (volume *A*).

Make a blank determination on the acetic anhydride-pyridine solution (volume *B*). Determine any free acid as described in the macroprocedure or by dissolving the sample in ethanol which has

Table I. Effect of Strength and Age of Acetic Anhydride-Pyridine Mixture on Macrodetermination of Hydroxyl in Dihydroxystearic Acid and Oleyl Alcohol

Sample	Acetylating Reagent		Age	% Hydroxyl		
	Ratio of anhydride to pyridine	Volume, ml.		Found	Average	
Dihydroxystearic acid ^a	1:7	3	Fresh	10.51	10.29	10.39
	1:7	6	Fresh	10.54	10.48	10.51
	1:7	3	4 days	10.38	10.12	10.25
	1:3	3	Fresh	10.83	10.78	10.81
Oleyl alcohol 1 ^b	1:3	3	4 days	10.88	10.74	10.81
	1:7	3	Fresh	5.20	5.46	5.33
	1:7	6	Fresh	6.25	6.24	6.25
	1:7	3	4 days	3.97	3.49	3.73
Oleyl alcohol 2 ^b	1:3	3	Fresh	6.30	6.31	6.31
	1:7	3	Fresh	5.60	5.34	5.47
	1:7	3	4 days	4.59	4.10	4.34
	1:3	3	Fresh	6.34	6.29	6.31
	1:3	3	4 days	6.55	6.40	6.36

^a Theoretical % OH = 10.75; weight of sample = 0.2 gram.

^b Theoretical % OH = 6.34; weight of sample = 0.8 gram.

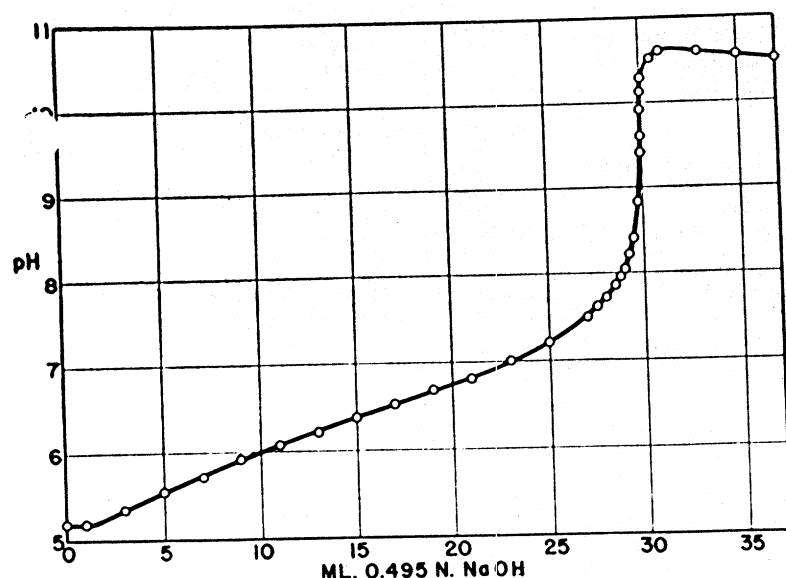


Figure 3. Titration Curve for Reaction Mixture

Table II. Macrodermination of Hydroxyl

Sample	Indicator Method			Potentiometric Method			Theoretical %
	1 %	2 %	Av. %	1 %	2 %	Av. %	
Dihydroxystearic acid	10.78	10.73	10.76	10.72	10.81	10.77	10.75
Monohydroxystearic acid	5.60	5.58	5.59	5.70	5.62	5.66	5.69
Oleyl alcohol 1	6.80	6.31	6.31	6.34
Oleyl alcohol 2	6.34	6.39	6.37	6.34
Methyl ricinoleate	5.47	5.62	5.55	5.44
Cyclohexanol ^a (Eastman Kodak White Label)	16.45	16.55	16.50	16.58	16.58	16.58	16.81
Benzyl alcohol ^a (Eastman Kodak White Label)	15.36	15.35	15.36	15.35	15.37	15.36	15.73
No. 4588 (dark colored) ^b	2.40	2.76	2.58	2.50	1.96	2.01	...
No. 5565 (dark colored) ^c	1.61	1.51	1.56	...
No. 5687 (dark colored) ^b

^a Not further purified.

^b Residue left in pot after distillation of methyl esters of fatty acids.

^c Residue left in pot after distillation of an oxidized oleic acid polymer.

been previously neutralized (mixed indicator), and titrating with 0.1 *N* alcoholic sodium hydroxide, using a rubber dam. From this titration calculate the volume of 0.1 *N* sodium hydroxide equivalent to the free acid in a 1-gram sample (volume *C*). The calculations are the same as for the macroprocedure.

DISCUSSION

The titration curve for alcoholic alkali versus acetic acid in a pyridine-water-*n*-butanol-ethanol solution is shown in Figure 3. The point of color change for the mixed indicator in this solution was at pH 9.8. Since the vertical portion of the curve extends between 9.2 and 10.3, a pH of 9.8 was selected for the potentiometric end point to make the potentiometric and indicator procedures interchangeable.

As indicated in Table I, both the age and the strength of the acetic anhydride-pyridine acetylating solution are important. The reagent made by mixing 1 volume of acetic anhydride with 7 volumes of pyridine gave theoretical results and remained effective for at least 4 days. A 1 to 7 acetic anhydride-pyridine solution was so dilute that it resulted in incomplete acetylation, as shown by the data for dihydroxystearic acid and oleyl alcohol. As this reagent aged, it became less effective as an acetylating agent, giving still lower results. The 1 to 3 acetic anhydride-pyridine solution permits the use of reagent grade pyridine with-

out its further purification, since a sufficient excess of the acetic anhydride is assured for complete acetylation of the sample, even though some has been consumed by moisture or other impurities in the pyridine.

For colorless solutions, identical values were obtained by the indicator method and the potentiometric method, as shown in Table II. Good precision was obtained with both methods. The cyclohexanol and benzyl alcohol, Eastman Kodak Company White Label reagents, were not further purified. The compounds for which theoretical values are cited were established as pure by such physical and chemical constants as iodine value, neutralization equivalent, saponification equivalent, melting point, and carbon and hydrogen analysis.

In the semimicroprocedure, it was necessary to carry out the reaction under water-cooled condensers to prevent the loss of small amounts of acetic anhydride during the acetylation. This loss also occurred in the macroprocedure, but here it was so small that it was not detected by titration with 0.5 *N* alkali.

The longer hydrolysis time required in the semimicroprocedure for samples containing organic acids was probably due to the formation of small amounts of mixed anhydrides, which are more difficult to hydrolyze than is acetic anhydride. High semimicro hydroxyl values were obtained with the 2-minute hydrolyses, probably owing to the failure to hydrolyze these mixed anhydrides completely. Since high values were not obtained by the macroprocedure, it was assumed that only small amounts of mixed anhydride were formed in either the semimicro or the macro methods and that the amount unhydrolyzed after the 2-minute heating period was not significant in the macroprocedure.

To obtain accurate hydroxyl values in the semimicroprocedure, all solutions must be protected from carbon dioxide during the titrations. This can best be done by covering the flask with a rubber dam and inserting the tip of the buret through a pinhole in the dam.

Table III presents a comparison of the values obtained by the semimicro- and macroprocedures.

Groups such as primary and secondary amines, and sulfhydryl, which contain active hydrogen and form acetylated products not hydrolyzed by hot water, interfere in the analysis. Comparison of the method of Mitchell, Hawkins, and Smith (7) for determining primary and secondary amines with the one herein described indicates that it may be possible to adapt this hydroxyl method to the determination of these amines and other interfering substances of the type noted above. Any compound which undergoes condensation to produce hydroxyl groups, such as aldehydes, interferes in the procedure described.

Table III. Determination of Hydroxyl by Semimicromethod

Sample	Semimicro Values			Average %	Macro Values %	Theory %
	1 %	2 %	3 %			
Dihydroxystearic acid	10.72	10.79	10.73	10.75	10.76	10.75
Oleyl alcohol 1	6.30	6.38	6.30	6.33	6.31	6.34
Partheniol	7.61	7.65	...	7.63	...	7.64
Cyclohexanol (Eastman Kodak White Label)	16.61	16.58	16.43	16.54	16.50	16.81
Benzyl alcohol (Eastman Kodak White Label)	15.33	15.29	15.46	15.36	15.36	15.73

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